NMU-induced mutation in *Aglaonema* by particle bombardment

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Abstract : *Aglaonema* is an ornamental plant which has beautiful leaf colour combination such as green and red; green and pink; green and white. Through NMU mutagenesis and particle bombardment technique, a new combination of leaf colour; red and white was obtained.. Sterile shoots of *Aglaonema* 1 week after subculture was used for shooting. These bombarded shoots elongated after 3 weeks of culture on Murashige and Skoog (MS) medium supplemented with 5.10-6 BAP. Mutated leaf of *Aglaonema* was obtained 4 months after shooting. There were 10 different types of mutations observed on leaf of *Aglaonema*, namely red blush, white, totally green, green and white, holed, wilting, green with a little white, green with white vascular, green, white and red, red and white. Red and white colour of *Aglaonema* leaves were obtained on 1, 2.5, 10, 250 and 500 and 2500 µM NMU.

Key words : *Aglaonema*, mutation, NMU, red and white leaves

Introduction

Floriculture has benefited tremendously from biotechnology and induced mutations, and there is a great potential to further improvement of ornamental plants with commercial values. Floriculture industry is quite profitable for exports in the international

market, and has a great impact on enhancing the socio-economic aspects in the developing countries. *Aglaonema* is an ornamental plant which has beautiful leaf colors. It has many combinations of color, such as green and red, green and white, green and pink, red, and so on. The beautiful combination leaf colors of *Aglaonema*, makes it well known in Asia, especially South East Asia. Variety of *Aglaonema* could be made by employing many methods; conventional and non conventional. Conventional method is conducted by breeders, crossing one variety or species with another and obtaining new variety through the seeds. In Non conventional method, the new varieties are Man-made, through technologies such as mutagenesis by a mutagen. .

Mutation is a natural phenomenon that refers to any change in the base sequence of DNA. The most common change is a substitution, addition, rearrangement, or a deletion of one of more bases. A mutagen is a physical agent or a chemical reagent that causes mutations. For example, nitrous acid react with some DNA bases, changing their identity and hydrogen-bonding properties. Mutagenesis is the process of producing a mutation. If it occurs in nature without the addition of a known mutagen, it is called spontaneous mutagenesis and the resulting mutations are spontaneous mutations. If a mutagen is used, the process is called induced mutagenesis [1]

A commonly used agent for inducing mutations (mutagenesis) in experimental organisms is nitrosomethylurea (NMU). NMU alkylates the DNA molecules frequently at N7 and O6 position of Guanine and sometimes at N1 and N3 position of Adenine and N1 of Cytosine. The alkylation of nitrogen bases may lead to mispairing and consequently transition or transversion type of point mutations [2] In the present study, NMU will be used as a chemical mutagen.

The purpose of this study was to create the red and white leaf of *Aglaonema*. To our knowledge the mutagenesis of *Aglaonema* induced by NMU using particle bombardment method is the first study and no one had done it before.

Material and Method

1. NMU solution preparation

The stock solution of NMU was 1 M. Dilution of NMU solution was performed as shown in table 1.

Table 1. Concentration of NMU

Dilution	Concentration
10x	1000 µM 2500 µM 5000 µM
100x	100 µM 250 µM 500 µM
1000x	10 µM 25 µM 50 µM
10000x	1 µM 2.5 µM 5 µM

2. Shooting by particle bombardment method

A scheme of particle bombardment process was shown in figure 1. A concentration of NMU solution (0.5 ml) was inserted into 1.5 ml microfuge tube. 2.5 mg of tungsten powder was poured into the NMU solution. The tube was then tapped with finger so that the tungsten powder and NMU were mixed.

6 µl of the mixed of tungsten powder and NMU was put into the hole of nillon pellet. The nillon pellet was then inserted into the focusing tube of particle gun. Thereafter, the bullet was inserted into the particle gun. The shoot of *Aglaonema* on the petri dish was put on the sample container. The experiment was repeated for the next sample and concentration of NMU. Each concentration was applied for 4 petri dishes, which 1 petri dish consisted of 10 shoots. For control, tungsten powder was only mixed with water and then shot to the shoot.

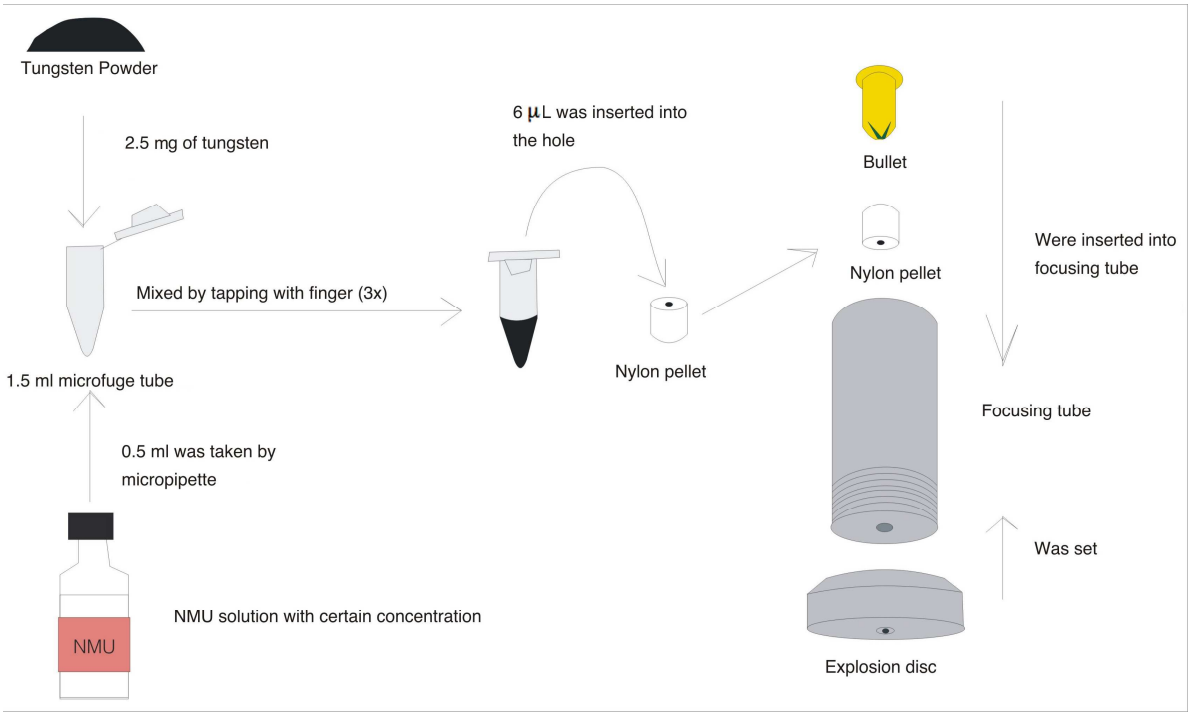

Fig 1. Scheme of NMU shooting by particle bombardment method

The shooting process was performed in the laboratory of Molecular Genetics, National Institute of Education, Nanyang Technological University, Singapore following the method by Chia et al [3] . After the shooting process finished, the cultures were put in the culture room for 3 days and brought to Indonesia for observation. The cultures were subcultured into hormone free media for 3 weeks. Thereafter, the cultures were subcultured into MS media supplemented with 5.10^{-6} BAP. After several subcultures, the mutation of *Aglaonema* was observed. Thereafter, the shoots were rooted onto MS medium containing 3 ppm IBA.

Result and Discussion

Shoots of *Aglaonema* were shot by NMU (Fig.2). After shooting, the shoots were nurtured on Murashige and Skoog (MS) medium supplemented with 5.10^{-6} M BAP.

The shoots then elongated as shown in fig.3 three months after shooting. One month later, the leaf of mutated *Aglaonema* expanded as shown in fig. 4. Small leaf was also developed on the mutated *Aglaonema*, the colour is red and white (Fig. 5).

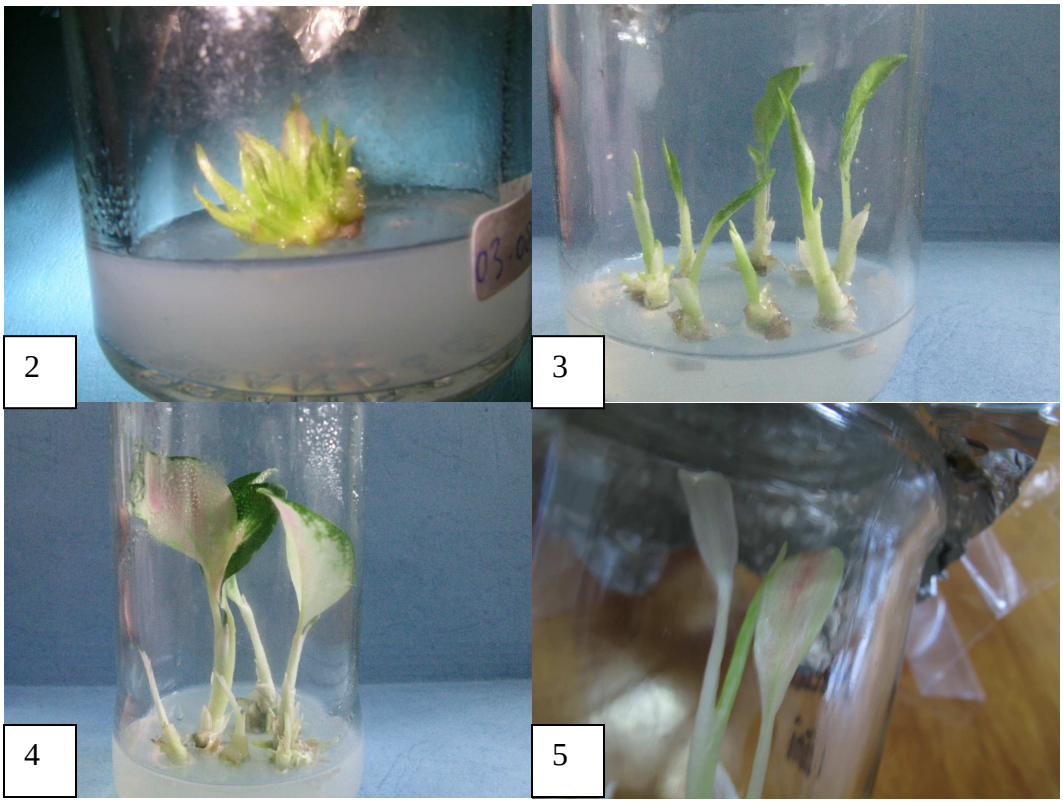

Fig. 2. Shoots of *Aglaonema* for mutagenesis experiment. Fig. 3. Elongated shoots 3 months after shooting. Fig.4. Mutated leaf of *Aglaonema* 4 months after shooting. Fig. 5. Red and white variegated leaf of *Aglaonema*.

There are 10 kinds of mutations observed in *Aglaonema* leaf as shown in fig. 6 namely, red blush; white; green, white and red; totally green; green and white; holed, wilting, green with a little white, green with white vascular, red and white. The red and white colour of *Aglaonema* leaf was the most wanted result in this study because the colour is similar to the colour of flag of Indonesia and Singapore. Therefore, the red and white *Aglaonema* will be used to celebrate the Indonesian independence day (on August 17) and Singapore independence day (August 9)), which is on that day red and white flag will be displayed in the whole country.

NMU alkylates the base of DNA molecules, frequently at N7 and O6 position of Guanine and sometimes at N1 and N3 position of Adenine and N1 of Cytosine. The alkylation of nitrogen bases may lead to mispairing and consequently transition or transversion type of point mutations [2]

Chlorophyll was synthesized through the biosynthesis of the glutamate amino acid [4] whereas the anthocyanin derived from phenylalanine amino acid [5] When the bases

of DNA was alkylated, it could lead to mispairing and did not synthesize glutamate amino acid in translation process. Therefore, the chlorophyll will not be synthesized and the leaf become albino or white. The anthocyanin was still synthesized so that the red colour was still seen in the leaf. It could explain why the red and white mutated leaf developed.

Glutamate amino acid was translated from codon GAA and GAG. The glutamate amino acid was derived from DNA transcription code CTT and CTC. When the Thymine was alkyated by NMU, the mRNA codon became GGG and GGG because the Thymine mispaired with Guanine. GGG codon will be translated as glycine amino acid [6] Therefore, glycine was synthesized instead of glutamate and no chloropyll will be formed.

The most common mutation induced by NMU in different plant species are variegations and albinos [2] These traits are maternally inherited which indicates that they reside in the plastid [7] Albino leaf was also observed in this study. The variegations are mainly caused by the presence of albino and green layers in the shoot apices [8]. The green and white variegations leaf was also found in this study. In majority of cases, this is resulted from spontaneous or induced mutations in chloroplasts DNA and in some cases by variable gene expression of nuclear DNA[9] or by transposable genetic elements [10] The albinos in most cases occur from changes in chloroplast DNA [11]

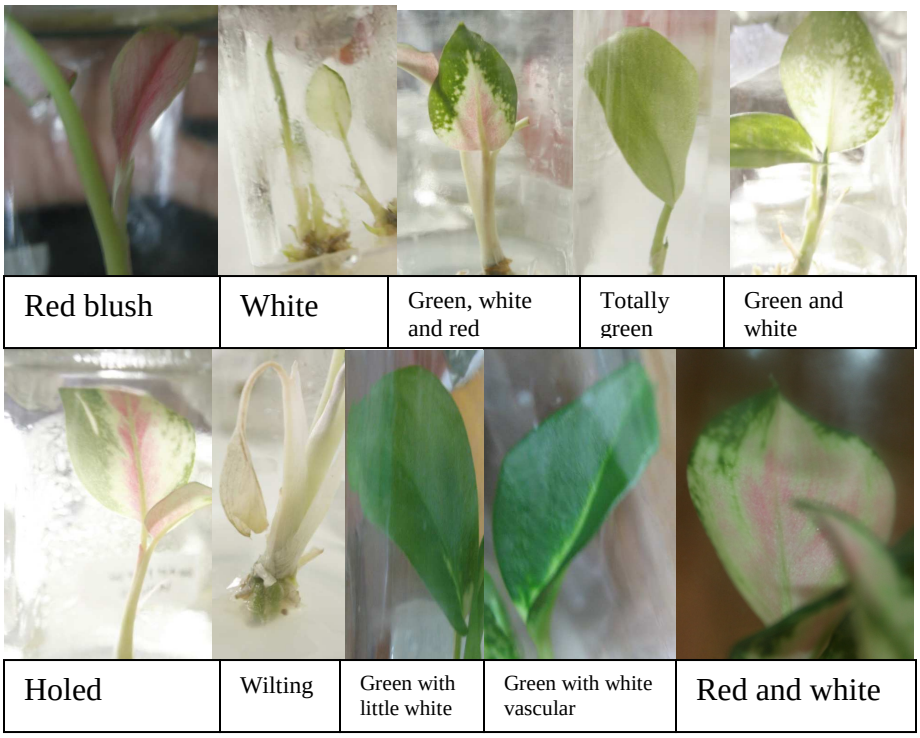

Fig. 6. Various kinds of mutated leaf of *Aglaonema* induced by NMU

Fig. 7 showed the effect of various concentration of NMU on mutated leaf of *Aglaonema*. The highest concentration of NMU, the highest amount of mutated leaf up to 250 µM. However, after 250 µM of NMU, the amount of mutation a little bit decreased. Red and white leaf colour were obtained on 1, 2.5, 10, 250 and 500 and 2500 µM.

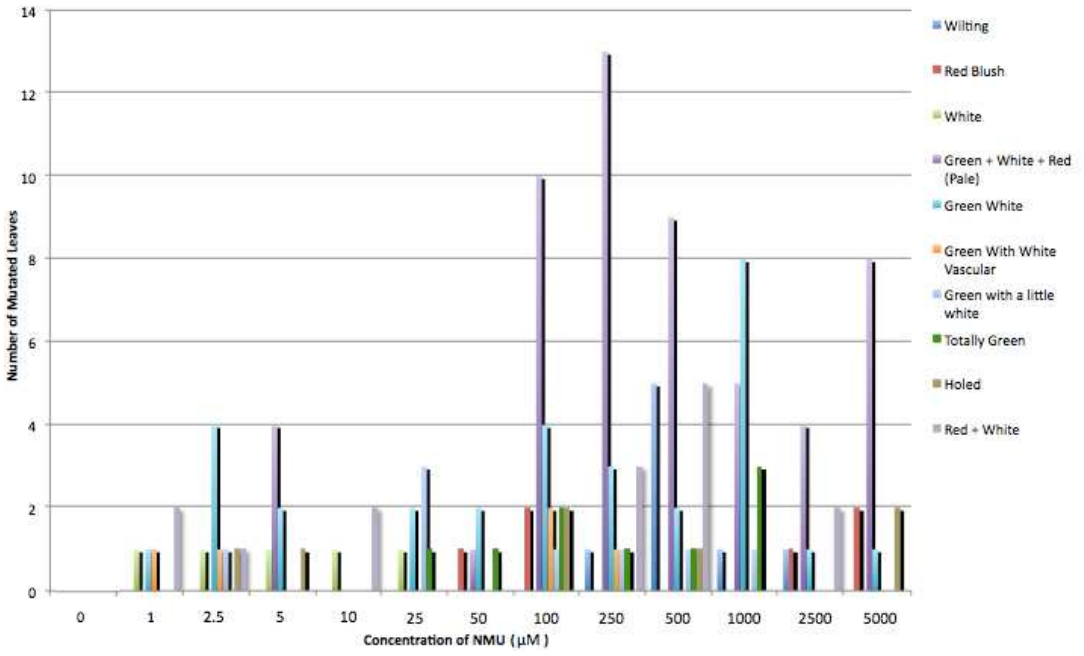

Fig. 7. Effect of various concentrations of NMU on mutated leaf of *Aglaonema*

Conclusion

In conclusion, we have shown that the particle bombardment method of mutagenesis is very efficient for Aglaonema and we were able to obtained 10 different types of new leaf coloration and pattern which are unique. In our studies, we have also confirmed that the optimal NMU concentration for the mutagenesis is 250 µM. These new Algaonema varieties have been potted out and are also propagated further in tissue culture for mass production.

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